

DOCKET NO: PHRM0028-101 (6195.NCN1)
Serial No.: 09/322,732

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IN THE SPECIFICATION:

Please replace the paragraph beginning on page 25, line 11 and ending on page 26, line 18 with the following paragraph

--Fluorescence measurements are carried out using an ISS Spectrofluorometer. The sample holder in the instrument is maintained at 26 [degrees] C using recirculated water from a constant temperature bath. Buffer solution consisting of 10 mM Tris-HCl pH 7.6, 10 mM MgCl₂, 50 mM NaCl, and 0.0001-0.001% ~~Tween-20~~ TWEEN-20™ (Polysorbate 20) is prepared. Other buffer systems such as HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid), MOPS (3-(N-Morpholino)propanesulfonic acid), Na-acetate, Na-phosphate may be used instead of Tris-HCl. The addition of Tween-20 or other nonionic detergent is necessary to prevent absorption of EFP to the walls of quartz cuvette. The buffer is passed through a 0.2 µm filter and degassed before use. Two milliliters of buffer are pipetted into a quartz cuvette (1 cm pathlength) containing a Teflon stir-bar. The quartz cuvette is placed into the temperature controlled sample holder of the spectrofluorometer. A solution of EFP protein (600 nM to 2.4 µM final protein concentration) is prepared by adding a specific volume of a stock solution of EFP protein to the cuvette containing 2 ml of buffer. A typical experiment uses 600 nM EFP, but data may be obtained with higher concentrations of protein. The cuvette containing the EFP solution is allowed to equilibrate with stirring for 10-15 minutes at 26C in the fluorometer. This time is required for equilibrium to occur between protein in solution and protein bound to the cuvette, and for the protein solution to reach the controlled temperature. A baseline fluorescence reading of EFP is obtained before adding any drug. The fluorescence of the single tryptophan residue (S. Aureus EFP) or three tryptophan residues (E. Coli EFP) are measured using an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Slit widths for excitation and emission wavelengths are 1 mm. Excitation wavelengths between 270-300 nm, emission wavelengths between 310-350 nm, and other slit widths may be used to measure the tryptophan fluorescence of EFP protein. A stock solution of drug (oxazolidinone or other test substance) is prepared in 100% DMSO. A typical experiment uses a 2 mM solution of drug in 100% DMSO. The drug is added in small increments (0.5-5.0 µl) and the fluorescence intensity at 330 nm is recorded after each addition. The final concentration of drug (oxazolidinone) is calculated after each drug addition. In a typical experiment, a total volume of 42 µl of 2 mM drug solution is added to give a final

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concentration of 42 μ Molar oxazolidinone. The intensity of the tryptophan fluorescence is plotted vs drug concentration and the data is fit the following equation:

$$F = \left[\frac{(F_0 - (F_0 - F_{00})) \times I}{I + K_d} \right]^{1/x}$$

where F is fluorescence intensity at 330 nm, F_0 is initial fluorescence without drug, F_{00} is the fluorescence intensity where the protein is saturated with drug, I is the drug concentration, K_d is the dissociation constant for the drug-protein interaction, and I is the correction factor for the inner filter effect. The oxazolidinones exhibit significant absorbance at the excitation wavelength (295 nm) so a correction for the inner filter effect of the drug is necessary. Goodness of fit of the experimental data to the theoretical curve is evaluated using the residual sum of squares.--